CAP 7C0211

ROCHE VITAMINS AND FINE CHEMICALS
HOFFMAN-LA ROCHE INC.

ASTAXANTHIN AS A PIGMENTER IN SALMON FEED

VOLUME 2 OF 7

Astaxanthin
Color Additive

INDEX

Teratology and Embryotoxicity Continued in Volume III

[redacted]

(D) ASTAXANTHIN:HUMAN FOOD SAFETY SUMMARY

(D)(1). Human Food Safety Summary

I. Acute Toxicity

The acute toxicity of astaxanthin was tested in mice and rats after oral or intraperitoneal application. At the doses tested, astaxanthin produced no lethality and no symptoms of toxicity.

Acute toxicity: Mouse orally (mg/kg)		
	24h	10 days
LD10	over 8000	over 8000
LD50	over 8000	over 8000
LD90	over 8000	over 8000

Acute toxicity: Mouse intraperitoneally (mg/kg)		
	24h	10 days
LD10	over 8000	over 8000
LD50	over 8000	over 8000
LD90	over 8000	over 8000

Acute toxicity: Rat orally (mg/kg)		
	24h	10 days
LD10	over 8000	over 8000
LD50	over 8000	over 8000
LD90	over 8000	over 8000

In another trial, the acute toxicity of astaxanthin in rats was determined following administration of 10 consecutive daily oral doses ranging from 125 to 2000 mg/kg b.w. There was no mortality and no symptoms of toxicity were reported.

Lethal doses in mg/kg p.o.	24 h after compound administration	24 h after 5 days of daily compound administration	24 h after 10 days of daily compound administration	10 days after 10 days of daily compound administration
LD 10	over 2000	over 2000	over 2000	over 2000
LD 50	over 2000	over 2000	over 2000	over 2000
LD 90	over 2000	over 2000	over 2000	over 2000

II. Mutagenicity

a) Ames Test

Astaxanthin at concentrations ranging from 0.03 to 5.0 mg/plate did not induce mutations in Salmonella typhimurium tester strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 with or without activation by a rat liver homogenate fraction.

b) Micronucleus Test

Astaxanthin in the form of the 10% gelatin beadlet was administered orally to mice at 30 hours and again at 6 hours prior to sacrifice. Dosage was 500, 1000, and 2000 mg/kg b.w. of the 10% gelatin beadlets. No compound-related increase in micronuclei was observed. Astaxanthin at the doses tested induces neither chromosome breaks nor mitotic disjunction in vivo.

III. Teratology and Embryotoxicity Studies

a) Rabbits

Astaxanthin was tested for embryotoxic and teratogenic effects in rabbits in accordance with the guidelines established by the American FDA and English CSM. Doses of 100, 200 and 400 mg/kg/day were administered orally to pregnant animals from day 7 to 19 inclusive of gestation. A control group received the vehicle for the same treatment period. All females were sacrificed at day 30 of gestation. Fetuses were removed by ovariohysterectomy, tested for viability (24 hrs.) and examined for macroscopic, skeletal and visceral and soft tissue anomalies.

The test compound was well tolerated by all females receiving treatment. During the course of the study there were neither overt signs of maternal sensitivity to the treatment nor significant changes in body weight development between the dams from the treated groups and those of the controls. In the low (100 mg/kg) and intermediate (200 mg/kg) dose groups the measured reproductive and litter parameters plus, course and outcome of pregnancy were unaffected, with all values comparing favorably to the concurrent controls. There was also no evidence of drug-related malformations among the examined fetuses. At the highest dose of 400 mg/kg, the only abnormal finding among the various measured reproductive parameters was a nominal increase in the incidence of resorptions (37.7%). However, this finding was not dose-related and failed to achieve statistical significance, due mainly

to the high resorption rate of the concurrent controls of 32.6%, and therefore, is not considered to represent a compound-related effect. There also was no evidence of teratogenic activity at the highest dose of 400 mg/kg.

Thus, it can be concluded that under the conditions of the present study, astaxanthin when administered orally to pregnant rabbits at doses of 100, 200 and 400 mg/kg/day is neither embryotoxic, teratogenic, nor has any effect on the course and outcome of pregnancy.

b) Rats

Astaxanthin was tested for embryotoxic and teratogenic effects in rats in accordance with the guidelines of the American FDA and English CSM. Doses of 0, 250, 500 and 1000 mg astaxanthin/kg/day were administered orally as a feed admix in form of gelatin beadlets from day 7 through 16 of gestation. No maternal death and no signs of maternal toxicity were noted in any of the dosage groups, with the exception of a dose dependent reduced weight gain during the treatment period.

There was no indication of any embryotoxic or teratogenic action of astaxanthin at any of the three dosage levels tested. The rearing experiment showed no indication of functional abnormalities in any of the dosage groups.

It can be concluded that under the conditions of this study, astaxanthin was neither embryotoxic nor teratogenic in rats at doses up to 1000 mg/kg/day.

IV. Reproductive Performance Study in Rats

Astaxanthin was tested in accordance with the guidelines of the American FDA and the English CSM for effects on fertility and general reproductive performance of the rat and on the in utero and postnatal development of the F1-offspring to time of weaning. The study includes the assessment of later development and of the reproductive capability of selected F1-offspring retained beyond weaning.

Doses of 25, 100 or 400 mg/kg/day were administered by oral gavage to 32 male rats/group, beginning 70 days prior to mating and continuing until sacrifice, and to 32 females/group beginning 14 days prior to mating and continuing through gestation until sacrifice or weaning. Control animals (32/sex) received the vehicle (rape-oil, 2 ml/kg) in a comparable regimen.

About half of the mated females in each group were sacrificed on about gestation day 14, while the remaining females were allowed to litter. F1-pups of selected litters were evaluated for developmental indices during lactation. On lactation day 23, selected weanlings were retained for learning and memory testing or the assessment of their reproductive capability.

The results of the study can be summarized as follows:

P-GENERATION

No substance-related mortality in males or females was observed in any of the dosage groups. The body weight gain of both P-males and P-females in all experimental groups matched that of concurrent controls.

The percentage of males which mated their partners, as well as the ratio of mated to pregnant females and the median precoital time were comparable between all groups.

Up to 400 mg/kg, the highest dose tested, the reproductive parameters of females sacrificed between gestation days 14 and 16 were within normal limits.

F1-GENERATION

In all experimental groups, the litter parameters such as the body weight gain of pups, the time of onset of developmental landmarks and the learning and memory ability matched that of the controls.

The neonatal mortality of the F1-generation in the highest dosage group (400 mg/kg) was at the upper limit of the biological range (25.6%). However, there was no statistical significance for this finding and no dose-relationship was evident. Therefore a substance-related impairment of pup viability was considered to be very unlikely.

In all dosage groups, the macroscopic and soft tissue examination of pups found dead during lactation showed isolated findings which were not considered to be substance-related. These included hematoma in the lung, empty stomach, and dilated renal pelvis and ureter. The gross examination of weanlings for malformations, as well as for liver and kidney weights, yielded normal findings. One neonate in the low-dose group (25 mg/kg) exhibited unilateral anophthalmia. This isolated anomaly was not considered to be substance-related.

The reproductive capability of F1-animals was not adversely affected in any of the experimental groups. The number of F2-pups which died or were cannibalized between lactation days 1 and 4 was unusually high in all groups, controls included, and, therefore, was considered to have resulted from other than substance-related influences.

CONCLUSION

It can be concluded from the results of this study that the no-effect-level of astaxanthin given by oral gavage to male and female rats during gametogenesis, mating, gestation and lactation was 400 mg/kg/day, the highest dose tested in this study.

V. 13-Week Tolerance Study in Rats

Astaxanthin in the form of gelatin beadlets was added to the feed of rats at concentrations of 6.25%, 12.5%, and 25% of beadlets. This corresponds approximately to an intake of 310, 620, and 1240 mg of astaxanthin/kg b.w./day, at the start of the study. Through the use of placebo beadlets, all groups, including the controls, received the same amount of beadlets in their feed. The concentrations of astaxanthin in the feed were kept constant during the whole study. Food wastage and avoidance of astaxanthin containing beadlets were minimal.

No astaxanthin related mortality occurred, body weights of dosed and control animals were similar.

Faeces of astaxanthin-fed animals were colored reddish. Yellow pigmentation of adipose tissue was observed at autopsy.

Focal to extensive alopecia with some tendency for reversal was observed in all groups.

There were no ophthalmoscopic findings related to the feeding of astaxanthin. Decreased organ weights in experimental groups were recorded for kidney (males, except at 6.25%), ovary (except at 6.25%), uterus, adrenals (females) and spleen (males and females). Histology of these organs was similar to the controls.

The hematology and blood chemistry parameters were within or close to the normal range, with the exception of decreased total serum proteins levels (week 13: males in 12.5% and 25% groups), and liver enzyme activities of several animals, which were sporadically increased, most probably as a result of parasite or Nosema infestation. Plasma cholesterol was elevated in all treated groups, but values were still within normal limits. Slight elevation of protein in urine (up to 200 mg/100 ml) was randomly found for animals of all groups with a number of cases in the 25% male group on week 13.

It can be concluded that the administration of astaxanthin at the above mentioned concentrations had been well tolerated and that no apparent toxicity, attributable to astaxanthin was observed.

VI. 13-Week Tolerance Study in Dogs

Astaxanthin in the form of gelatin beadlets containing 6.1% w/w of astaxanthin was administered for a period of 3 months to 3 groups of six dogs each (3 males + 3 females), at concentrations of 2.5%, 5% and 10%

w/w of beadlets by means of feed admix. Average astaxanthin intake for the 13-week period was 41, 76, and 162 mg/kg b.w./day for the 2.5%, 5%, and 10% groups, respectively.

Six additional dogs were kept as controls and received a feed admix containing placebo beadlets.

The test compound was well tolerated for the entire period of 3 months and did not cause any adverse effects or show any evidence of a systemic toxicity with regard to the general state of health, the body weight development, the behavior of the dogs, the hematological and clinical chemical parameters, the ophthalmoscopy, and the autopsy and histological appearance of the organs.